PhytoKeys 190: 69–85 (2022)
doi: 10.3897/phytokeys.190.78164
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Two new species of *Navicula* (Bacillariophyta) from Southeast Asia

Maxim S. Kulikovskiy[1], Dmitry A. Chudaev[2], Anton M. Glushchenko[1], Irina V. Kuznetsova[1], John Patrick Kociolek[3,4]

1 *K.A. Timiryazev Institute of Plant Physiology RAS, IPP RAS, 35 Botanicheskaya St., Moscow, 127276, Russia* **2** *Department of Mycology and Algology, Faculty of Biology, M.V. Lomonosov Moscow State University, Leninskie Gory 1, building 12, Moscow, 119234, Russia* **3** *Museum of Natural History, Boulder, Colorado, 80309, USA* **4** *Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado, 80309, USA*

Corresponding author: Maxim S. Kulikovskiy (max-kulikovsky@yandex.ru)

Academic editor: K. Manoylov | Received 19 November 2021 | Accepted 3 February 2022 | Published 18 February 2022

Citation: Kulikovskiy MS, Chudaev DA, Glushchenko AM, Kuznetsova IV, Kociolek JP (2022) Two new species of *Navicula* (Bacillariophyta) from Southeast Asia. PhytoKeys 190: 69–85. https://doi.org/10.3897/phytokeys.190.78164

Abstract

We present light and scanning electron microscopical observations on two new species of *Navicula* Bory sensu stricto from Southeast Asia. *Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek, **sp. nov.** differs from taxa with similar outline and size by the combination of simple drop-like external raphe endings deflected to the primary valve side, presence of well-developed external longitudinal grooves and relatively low lineolae density. *Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek, **sp. nov.** can be discriminated from the taxa of similar valve shape and size by consistently lower lineolae density, and from the majority of them also by the structure of external proximal raphe endings with small projections in proximal parts and larger triangular insertions in distal parts. Some remarks on *Navicula* species diversity and its distribution in the Southeast Asia are given.

Keywords

Diatoms, distribution, freshwaters, morphology, *Navicula*, new species, Southeast Asia, taxonomy

Introduction

This investigation continues our description of new species from the genus *Navicula* Bory from Southeast Asia. With both freshwater and marine representatives, the genus *Navicula* Bory is the most taxon-rich genus of all diatoms (Kulikovskiy et al. 2016).

Copyright *Maxim S. Kulikovskiy et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

However, the number of species included in this genus has actually been decreasing over the last 20 years mainly because of the description of new genera that include species formerly in in *Navicula*. The genus *Navicula* is easily distinguished from many other diatom genera by its lineolate uniseriate striae (Kulikovskiy et al. 2016). In some areas some morphologically unusual groups of species from the genus *Navicula* have been recognized, such as species with hyaline area on valve face. An example of this is *Navicula lacusbaicali* Skvortzov & Meyer from ancient Lake Baikal (Kulikovskiy et al. 2012). This group of *Navicula* formed species-flock (e.g. Kociolek et al. 2017); this is a group of closely-related taxa originated from single parent by explosive radiation (see Kociolek et al. 2019). New species from the genus *Navicula* are described often, indicating we are still gaining a better understanding of the real diversity in this genus. The taxonomy of some species in the genus is in need of clarification as well (Witkowski et al. 2010; Kulikovskiy et al. 2016).

Southeast Asia is a very interesting region for aquatic protist diversity, yet studies from the region are relatively few. More recently, we have been investigating diatoms and many other organisms of Southeast Asia (Glushchenko et al. 2021), including new genera and species, for example from Laos and Vietnam (e.g. Kulikovskiy et al. 2015, 2018, 2019; Glushchenko et al. 2016, 2017, 2018, 2019, 2020; Liu et al. 2018; Kezlya et al. 2020). A comprehensive review of diatom studies was summarized in Glushchenko et al. (2021). A few publications were dedicated to the diversity of new species from the genus *Navicula* (Chudaev et al. 2018; Kulikovskiy et al. 2020a,b, 2021) of Southeast Asia. Continuation of this work is important for understanding the diversity of the genus *Navicula* and diatoms as a whole from such an interesting region. Freshwater ecosystems from this region will need investigation in future for water quality assessment, using modern methods in ecological monitoring like DNA barcoding (Rimet et al. 2019). But a primary understanding of the biodiversity of the region is a necessary first step for future investigations and applying new methods.

The aim of this publication is to present a morphological investigation using light and scanning electron microscopy to describe two new diatom species of the genus *Navicula* from Southeast Asia.

Materials and methods

Samples from Vietnam were collected by M.S. Kulikovskiy and E.S. Gusev during expeditions organized and permitted by the Joint Russian-Vietnam Tropical Centre, Ecolan 1.2 and 3.2 projects. The sample from Laos was collected by E.L. Konstantinov during an expedition of Kaluga and Laos Joint Universities (Russia and Laos).

A list of all samples examined in this study with their geographic positions is presented in Table 1. Water mineralization and temperature measurements were performed using the Hanna Combo (HI 98129) device, Hanna Instruments, Inc., USA. Material was collected with a pipette into 15- and 50-ml polymer test tubes. Samples were fixed with 37% formaldehyde.

The samples were treated with 10% hydrochloric acid to remove carbonates and washed several times with deionized water for 12 h. Samples were subsequently boiled in concentrated hydrogen peroxide (≈ 37%) to dissolve organic matter. They were washed again with deionized water four times at 12 h intervals. After decanting and filling with deionized water up to 100 ml, the suspension was spread onto coverslips and left to dry at room temperature. Permanent diatom preparations were mounted in Naphrax (refraction index = 1.73). Light microscopic (LM) observations were performed with a Zeiss Axio Scope A1 microscope equipped with an oil immersion objective (× 100, n.a. 1.4, differential interference contrast [DIC]) and Axiocam ERc 5s camera (Zeiss). Valve ultrastructure was examined by means of scanning electron microscope JEOL JSM-6380LA (JEOL Ltd., Japan) operating at 20 kV and 8 mm working distance (Faculty of Biology, M.V. Lomonosov MSU, Moscow). For scanning electron microscopy (SEM), parts of the suspensions were fixed on aluminium stubs after air-drying. The stubs were sputter coated with 50 nm of gold in an Eiko IB 3.

Fixed material and slides are deposited in the collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia. Isotypes are deposited in Diatom collection of the Department of Mycology and Algology, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia.

Results

Navicula winoniformis **Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov.**
Figs 1, 2

Navicula gottlandica sensu Lee, 2012 (fig. 4E) (Algal Flora of Korea vol. 3, num. 8).

Holotype. Slide 03572 in collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, represented here by Fig. 1E.

Isotype. Slide MW-D 898s1 in Diatom collection of the Department of Mycology and Algology, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia.

Type locality. Vietnam. Khánh Hòa Province, Suối Dầu Reservoir, benthos, 12°09.900'N, 109°03.200'E, 36 m elev., leg. E.S. Gusev, 10.08.2010.

Description. LM (Fig. 1A–P). Valves narrowly lanceolate with rostrate to subcapitate apices, length 29.7–49.0 μm, width 6.5–8.0 μm (n=32). Axial area narrow, slightly widening towards valve centre, central area transversely expanded, with irregular border due to unequal shortening of central striae, usually occupying about 1/2 of valve width. Striae radiate, becoming strongly convergent at the valve ends, 12–14/10 μm. Lineolae easy to resolve in light microscope, 24–27/10 μm. Raphe filiform to narrowly lateral, terminal fissures deflected to the secondary valve side, central pores straight or very slightly deflected to primary valve side, not close standing.

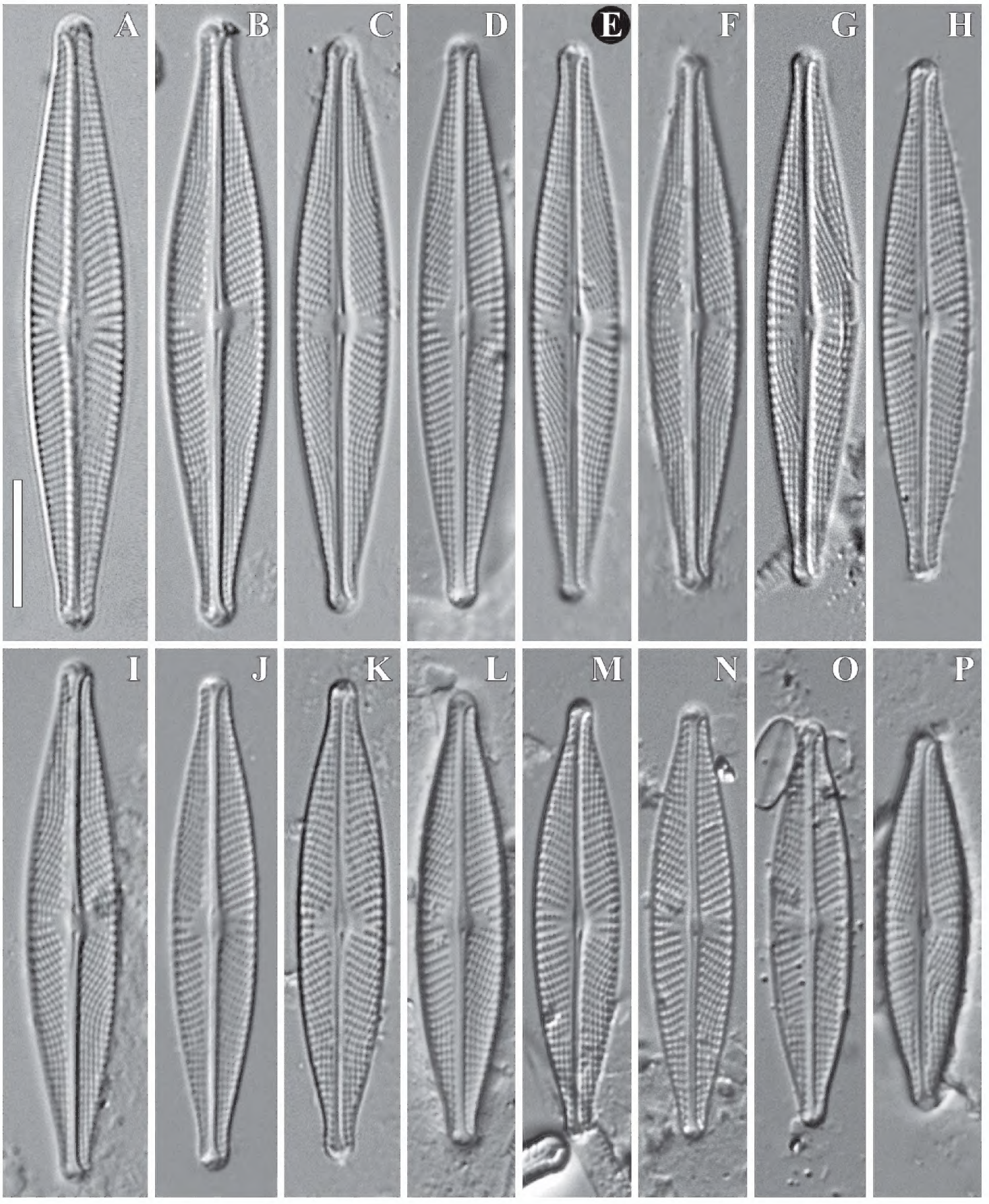

Figure 1. A–P *Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov. LM, DIC. Size diminution series. Vietnam, slides no 00269 (**A**), 00318 (**L, P, N**), 00323 (**I**), 00326 (**C**), 00328 (**B**), 02079 (**O**), 03572 (**E, F, H, K, M**), 03773 (**D**), 04853 (**J**). Laos, slide no 00956 (**G**). Holotype (**E**). Scale bar: 10 μm.

SEM, external view (Fig. 2A–C). Areola openings apically elongate, lying in distinct longitudinal grooves (Fig. 2C, white arrow). Four small isolated areolae with almost circular openings present at valve apices on valve mantle (Fig. 2C, white arrowheads). Raphe-sternum elevated very slightly in valve centre (Fig. 2B, black arrow). Central pores drop-like without any projections, deflected slightly to primary

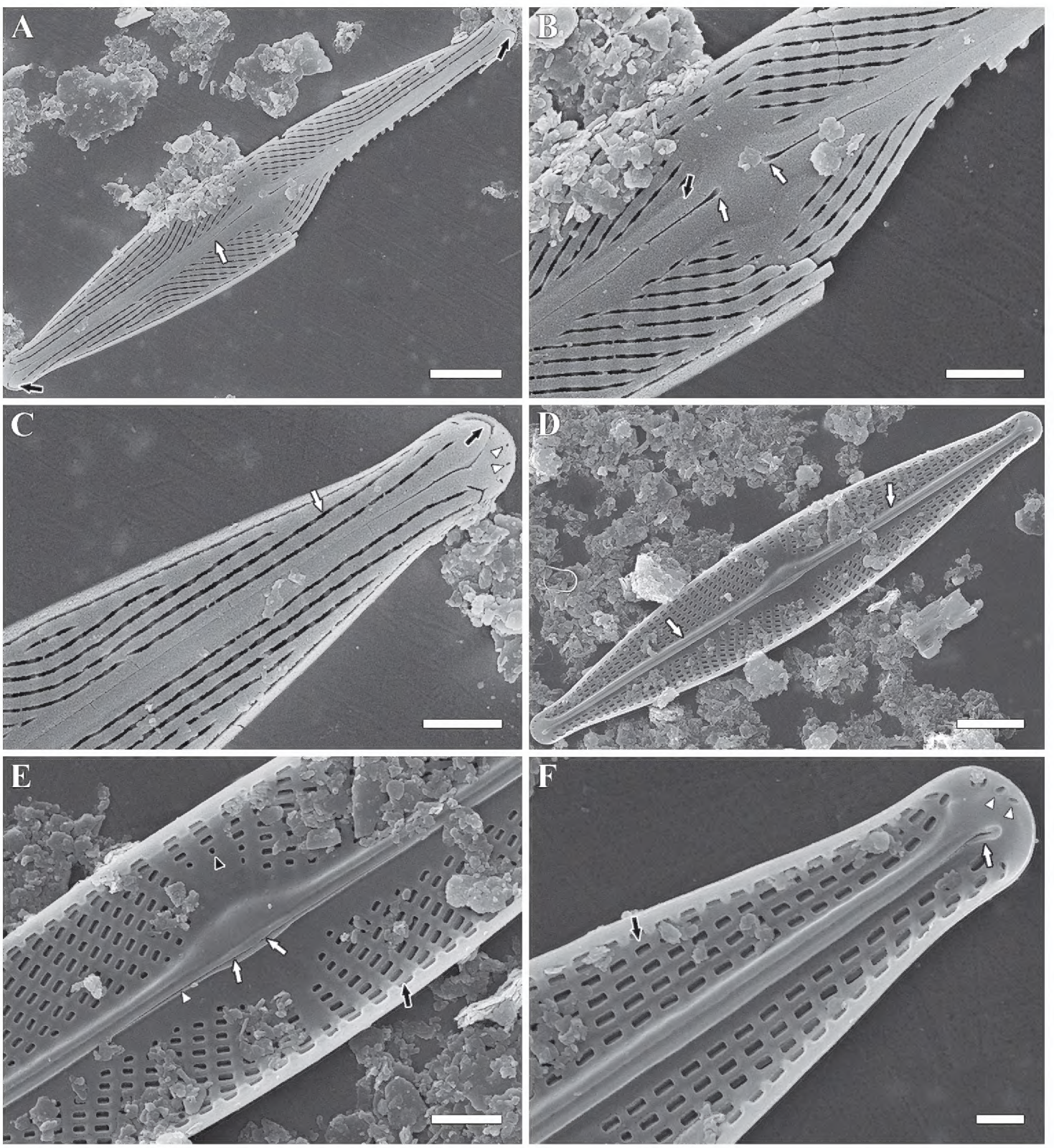

Figure 2. A–F *Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov. SEM. Sample no 03572. **A–C** external views **D–F** internal views **A, D** the whole valve **B, E** central area **C, F** valve end. **A** black arrows show the terminal raphe fissures. White arrow shows the indistinct kink of raphe branch **B** white arrows show central raphe pores. Black arrow shows the very slightly elevated raphe-sternum **C** black arrow shows the terminal raphe fissure. White arrow shows the longitudinal grooves where areola openings are located. White arrowheads show the small isolated apical areolae **D** white arrows show the accessory rib **E** black arrow shows the rectangular areola opening. Black arrowhead shows the circular areola opening. White arrows show the central raphe endings. White arrowhead shows the raphe slit **F** black arrows show the stria which are slightly wider than virgae. White arrow shows the helicroglossa. White arrowheads show the small isolated apical areolae. Scale bars: 5 μm (**A**); 2 μm (**B–E**); 1 μm (**F**).

valve side (Fig. 2B, white arrows). Terminal fissures hooked to secondary valve side (Fig. 2A, C, black arrows). Raphe branches with indistinct kink in proximal parts (Fig. 2A, white arrow).

SEM, internal view (Fig. 2D–F). Striae slightly wider than virgae, lie at same level as latter, no transapical grooves formed (Fig. 2E, F, black arrows). Internal areola openings rectangular (Fig. 2F, black arrow) or circular (near central area) (Fig. 2E, black arrowhead), wider than external ones. Hymenes were not preserved during the material treatment. Four to five small isolated areolae present at each apex (Fig. 2F, white arrowheads). Raphe slit opens obliquely to secondary side and visible in proximal (Fig. 2E, white arrowhead) and distal (Fig. 2F, white arrow) parts only. Raphe-sternum very narrow, widened in centre, separated from accessory rib by longitudinal groove. Accessory rib well-developed (Fig. 2D, white arrows), widened unilaterally in valve centre and at valve apices. Central raphe endings straight, simple, connected by thin indistinct furrow (Fig. 2E, white arrows). Distal raphe endings well-developed helictoglossae deflected to secondary valve side (Fig. 2F, white arrow).

Etymology. Specific epithet is given due to similarity of new species to *Navicula winona* Bahls.

Distribution. Vietnam. Type locality (Suối Dầu Reservoir, slide no 03572). Suối Dầu River (slide no 00318, 00323, 03773), Suối Tiên River (slide no 00326, 00328), Cái River (slide no 04853), Mường Hoa River (slide no 02079), Da Tien Reservoir (slide 02069). Laos, Nam Lik River (slide no 00956).

Ecology. *Navicula winoniformis* sp. nov. was found in a the reservoirs, and in rivers and waterfalls with different conductivity and pH values (Table 1). Below are the dominant species found in the samples along with *N. winoniformis* sp. nov.:

Sample 03572: *Neidium gracile* Hustedt, *Encyonopsis fonticola* (Hustedt) Krammer. Sample 00956: *Oricymba voronkinae* Glushchenko, Kulikovskiy & Kociolek, *Pinnularia sikkimensis* S.K. Das, C. Radhakrishnan, Kociolek & Karthick, *Pinnularia stricta* Hustedt, *Luticola muticoides* (Hustedt) D.G. Mann.

Sample 00318: *Hydrosera triquetra* Wallich, *Diadesmis confervacea* Kützing, *Luticola nipkowii* (Meister) Glushchenko & Kulikovskiy.

Sample 00323: *Gomphonema dalatica* Glushchenko, Kulikovskiy & Kociolek, *Eunotia indosinica* Glushchenko & Kulikovskiy.

Sample 03773: *Eunotia indomalaica* Glushchenko, Kulikovskiy & Kociolek, *O. voronkinae*.

Sample 00326: *Gomphonema subventricosum* Hustedt, *D. confervacea*, *Oricymba perjaponica* (Krammer & Lange-Bertalot) Kulikovskiy, Glushchenko & Kociolek, *Rhopalodia gibba* (Ehrenberg) O. Müller, *Encyonema javanicum* (Hustedt) D.G. Mann.

Sample 00328: *Placoneis paraundulata* T. Ohtsuka, *E. javanicum*, *Eunotia laoarcus* Glushchenko, Kulikovskiy & Kociolek.

Sample 04853: *D. confervacea*, *Platessa oblongella* (Østrup) Wetzel, Lange- Bertalot & Ector, *Luticola taylorii* Levkov, Metzeltin & Pavlov, *N. gracile*, *E. javanicum*, *O. voronkinae*, *Frustulia magaliesmontana* Cholnoky.

Sample 02079: *P. oblongella*, *Reimeria sinuata* (Gregory) Kociolek & Stoermer, *E. javanicum*, *Encyonema leei* Krammer.

Sample 00269: *G. dalatica*, *Adlafia lamdongiensis* Glushchenko, Kulikovskiy & Kociolek, *Kobayasiella lamii* Glushchenko, Kulikovskiy & Kociolek.

Table 1. List of samples examined in this study. Geographic locality of samples and measured parameters indicated.

Slide	Locality	Habitat	Coordinates	Altitude, m	t, °C	pH	Conductivity, μS cm^{-1}	Coll. date
			Vietnam					
00269	Lâm Đồng Province, Da Tien Reservoir	benthos	11°58.816'N, 108°26.987'E	1503	21.5	6.4	81	21.06.2012
00318 00323	Khánh Hòa Province, Suối Dầu River	benthos periphyton	12°06.768'N, 108°59.888'E	275	24	6.7	92	02.07.2012
00326 00328	Khánh Hòa Province, Suối Tiên River	benthos periphyton	12°12.199'N, 109°01.694'E	68	26	6.9	101	02.07.2012
02079	Lào Cai Province, neat the Sa Pa Town, Mường Hoa River	periphyton	22°15.415'N, 103°8.883'E	887	25.5	8.4	204	10.05.2015
03572	Khánh Hòa Province, Suối Dầu Reservoir	benthos	12°09.900'N, 109°03.200'E	36	31.4	7.3	84	10.08.2010
03773	Khánh Hòa Province, Suối Dầu River	periphyton	12°06.768'N, 108°59.891'E	275	25	6.7	119	28.05.2012
04853	Khánh Hòa Province, Cái River	plankton	12°15.983'N, 109°06.517'E	13	31.6	7.0	40	17.04.2010
			Laos					
00956	Vientiane Province, Van Vieng District, Nam Lik Village, Nam Lik River	benthos	18°36.808'N, 102°24.605'E	196	23.5	6.9	98	24.11.2011
00962	Champasak Province, Bolaven Plateau, near the Pakse Town, unnamed waterfall	benthos	15°16.616'N 106°19.935'E	1149	24.5	6.9	84	30.11.2011
01621	Champasak Province, Bolaven Plateau, near the Paksong Town, Tad Yueang Waterfall	benthos	13°57.266'N 105°54.890'E	78	26.2	7.1	96	01.12.2011

***Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov.**
Figs 3, 4

Holotype. Slide 00962 in collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, represented here by Fig. 3D.

Isotype. Slide MW-D 899s1 in Diatom collection of the Department of Mycology and Algology, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia.

Type locality. Laos. Champasak Province, Bolaven Plateau, near the Pakse Town, unnamed waterfall, benthos, 15°16.616′N, 106°19.935′E, 1149 m elev., leg. E.L. Konstantinov, 30.11.2011.

Description. LM (Fig. 3A–N). Valves lanceolate with acutely rounded, non-protracted apices, length 33.9–56.5 μm, width 7.7–9.1 μm (n=31). Axial area narrow, widening towards valve centre, slightly wider on the secondary valve side, central area more or less round, occupying 1/3–1/2 of valve width, margins of raphe-sternum clearly visible as longitudinal lines at the central area. Striae radiate, sometimes (usually in larger valves) becoming parallel at the valve ends, 9.3–10.5/10 μm. Lineolae easy to resolve in light microscope, 17.5–19.7/10 μm. Raphe filiform to narrowly lateral, terminal fissures barely visible, deflected to the secondary valve side, central pores deflected to secondary valve side.

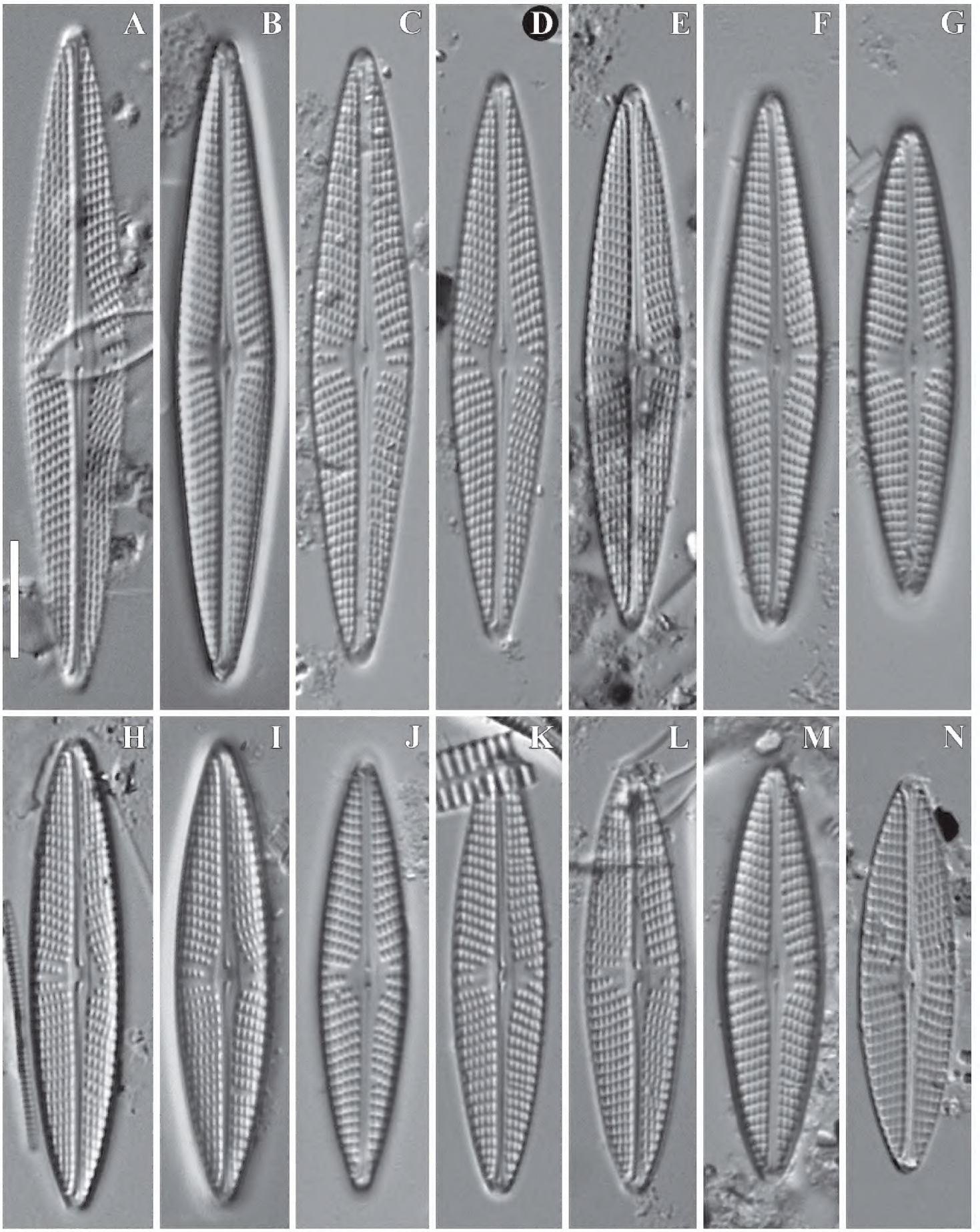

Figure 3. A–N *Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov. LM, DIC. Size diminution series. Laos, slides no 00962 (**A, B, F, C–E, G, I, J, L, M, N**), 01621 (**H, K**). Holotype (**D**). Scale bar: 10 μm.

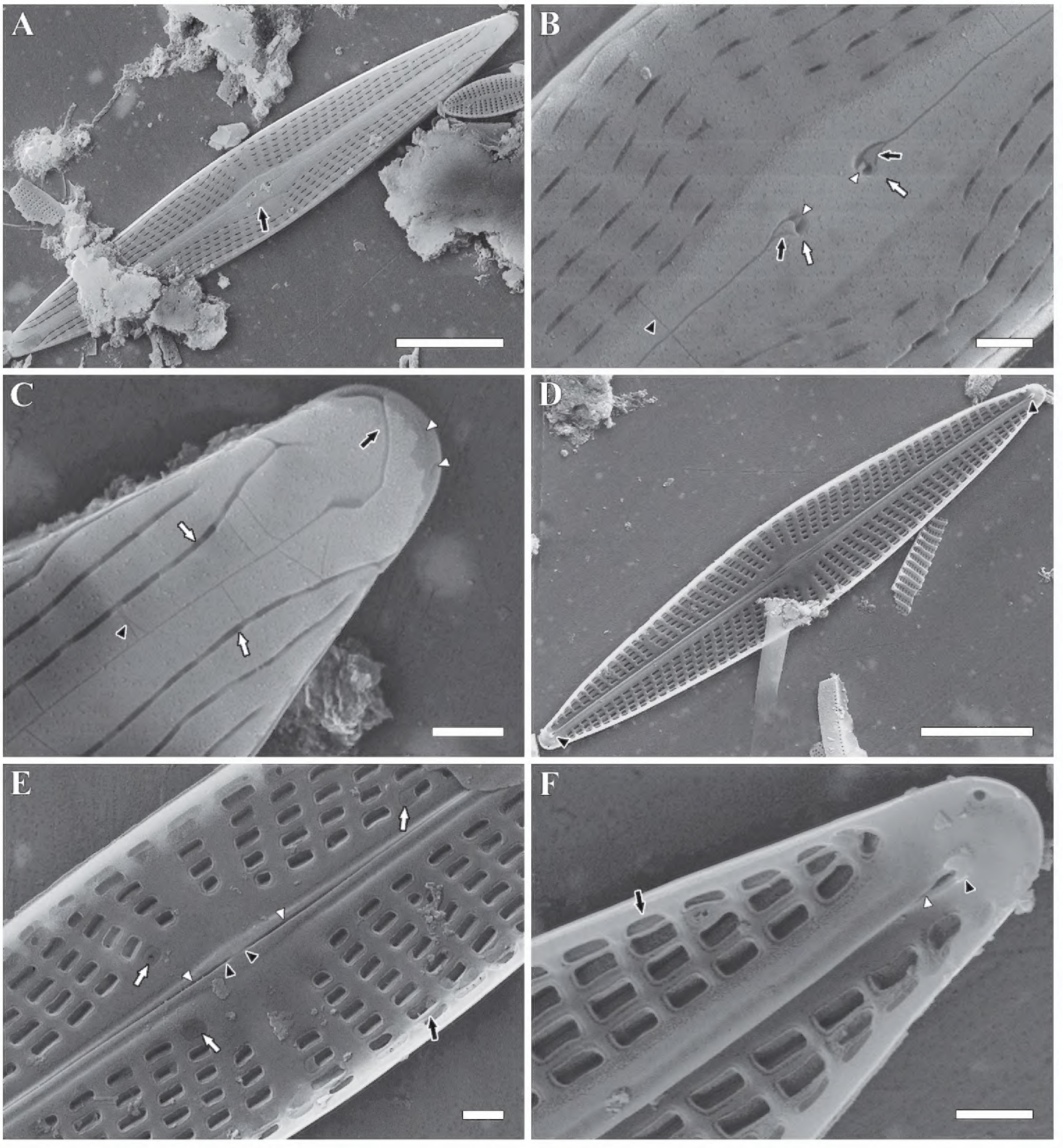

Figure 4. A–F *Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek, sp. nov. SEM. Sample no 00962. **A–C** external views **D–F** internal views **A–C** external views **D–F** internal views **A, D** the whole valve **B, E** central area **C, F** valve end **A** black arrow shows the very slightly elevated raphe-sternum **B** white arrows show the drop-like central pores. Black arrows show the larger triangular insertion in distal part. White arrowheads show the small projections in proximal part. Black arrowhead shows the transapical sutures **C** black arrow shows the terminal raphe fissure. White arrows show the longitudinal grooves where areola openings are located. Black arrowhead shows the transapical sutures. White arrowheads show the small isolated apical areolae **D** black arrowheads show the helictoglossae **E** black arrow shows the stria which slightly wider than virgae. White arrows show the areola openings occluded with hymenes. Black arrowheads show the central raphe endings. White arrowheads show the raphe slits **F** black arrow show the stria which are wider than virgae. Black arrowhead shows the helictoglossa. White arrowhead shows the raphe slit. Scale bars: 10 μm (**A, D**); 1 μm (**B, C, E, F**).

SEM, external view (Fig. 4 A–C). Areolae apically elongate, areolae openings lie in shallow apical grooves more clearly expressed near valve apices (Fig. 4C, white arrows). At each apex two small isolated apical areolae present with shorter slits oriented obliquely or subparallel to valve margin (Fig. 4C, white arrowheads). Raphe-sternum elevated slightly above valve surface in centre, widened, with asymmetrical margins (Fig. 4A, black arrow). Its primary margin slightly convex, secondary margin slightly concave or straight. Central pores drop-like, deflected to secondary side. Each pore possesses small projection in proximal part (Fig. 4B, white arrowheads) and larger triangular insertion in distal part (Fig. 4B, black arrows). Terminal fissures hooked to secondary side (Fig. 4C, black arrow), proximal parts of fissures dilated. On axial area are present thin transapical sutures (Fig. 4B, C, black arrowheads).

SEM, internal view (Fig. 4D–F). Striae wider than virgae, lie at same level as latter, no transapical grooves formed (Fig. 4E, F, black arrows). Internal areola openings occluded with hymens (mostly destroyed) rectangular, wider than external ones (Fig. 4E, black arrows). Raphe slit opens obliquely to secondary side and visible in proximal and distal parts only (Fig. 4E, F, white arrowheads). Raphe-sternum very narrow, widened in centre, flanked with apical grooves from both sides. Accessory rib almost absent, especially in proximal part of valve. Central raphe endings straight, simple (Fig. 4E, black arrowheads). Distal raphe endings well-developed straight helictoglossae (Fig. 4D, F, black arrowheads).

Etymology. The specific epithet refers to the comparatively low density of areolae in the new species.

Distribution. Laos. Type locality (unnamed waterfall, slide no 00962) and Tad Yueang Waterfall (slide no 01621).

Ecology. *Navicula sparsilineolata* sp. nov. was found in a the waterfalls with low conductivity and circumneutral pH values (Table 1). Below are the dominant species found in the samples along with *N. sparsilineolata* sp. nov:

Sample 00962: *Gomphonema capitatum* Ehrenberg, *D. confervacea*, *Luticola burmensis* Metzeltin & Levkov, *L. nipkowii*, *P. sikkimensis*.

Sample 01621: *L. burmensis*, *L. nipkowii*, *P. sikkimensis*, *Platessa oblongella* (Østrup) Wetzel, Lange- Bertalot & Ector.

Discussion

The new species *Navicula winoniformis* sp. nov. is similar to a few previously described species. These include *N. cryptocephala* Kützing, 1844, *N. krammerae* Lange-Bertalot, 1996, *N. densilineolata* (Lange-Bertalot) Lange-Bertalot, 1993, *N. wildii* Lange-Bertalot, 1993, *N. insulsa* Metzeltin & Lange-Bertalot, 1998, *N. oetzvallensis* Lange-Bertalot in Werum & Lange-Bertalot, 2004, *N. winona* Bahls, 2012 and *N. praeterita* Hustedt, 1945. A summary comparison of these taxa is presented in Table 2.

Table 2. Morphometric features of *Navicula winoniformis* Chudaev, Glushchenko, Kulikovskiy & Kociolek sp. nov. and comparison with similar taxa.

Taxon	Outline	Valve ends	Valve length, µm	Valve width, *µm*	Striae in 10 µm	Areolae in 10 µm	References
N. winoniformis sp. nov.	narrowly lanceolate	rostrate to subcapitate	29.7–49.0	6.5–8.0	12–14	24–27	This study
N. cryptocephala	lanceolate to narrowly lanceolate	gradually narrowing or weakly rostrate, subcapitate to obtusely rounded	20–40	5–7	14–18	40–44	Lange-Bertalot 2001; Jüttner et al. 2020
N. krammerae	lanceolate	protracted and beak-like, neither distinctly nor acutely rounded	28–36	6.0–7.5	13–14	28–31	Lange-Bertalot 2001
N. densilineolata	narrowly lanceolate	almost acutely rounded	28–60	6.0–7.5	10–13	27–30	Lange-Bertalot 2001
N. wildii	(narrow) lanceolate	gradually narrowing to obtusely rounded, very rarely almost imperceptibly protracted	23–50	5.5–7.5	11.0–12.5	ca. 35	Lange-Bertalot 2001; Kulikovskiy et al. 2016
N. insulsa	rather linear-lanceolate	protracted, subcapitate to obtusely rounded	40–54	6–7	14–16	25–27	Metzeltin and Lange-Bertalot 1998
N. oetzvallensis	narrowly lanceolate to linear-lanceolate	weakly protracted	30–44	7.0–7.5	10.5–12.5	28–30	Werum and Lange-Bertalot 2004
N. winona	narrowly lanceolate	gradually attenuated and very narrow subcapitate	39–52	7.2–8.2	12–13	24–28	Bahls 2012
N. praeterita	lanceolate	rostrate-subcapitate	25–40	5.5–8.5	12–14	22–25	Lange-Bertalot 2001

Table 3. Morphometric features of *Navicula sparsilineolata* Chudaev, Glushchenko, Kulikovskiy & Kociolek sp. nov. and comparison with similar taxa.

Taxon	Outline	Valve ends	Valve length, µm	Valve width, *µm*	Striae in 10 µm	Areolae in 10 µm	References
N. sparsilineolata sp. nov.	lanceolate	acutely rounded, non protracted	33.9–56.5	7.7–9.1	9.3–10.5	17.5–19.7	This study
N. pseudolanceolata	lanceolate to rhombic-lanceolate	gradually narrowed	28.7–50.0	7.0–9.8	9.5–11.2	22.0–25.7	Lange-Bertalot 2001; Chudaev and Gololobova 2016
N. oppugnata	lanceolate to linear-lanceolate	usually obtusely rounded	30–60	8.5–12.0	7–12	ca. 24	Lange-Bertalot 2001
N. johncarteri	lanceolate	gradually narrowing to a wedge, neither distinctly acutely nor obtusely rounded	40–75	9–12	8–10	ca. 25	Lange-Bertalot 2001
N. trophicatrix	lanceolate to rhombic-lanceolate	gradually narrowing to a wedge, neither distinctly acutely nor obtusely rounded	25–50	7.5–10.0	11–13	21–24	Lange-Bertalot 2001
N. sancti-naumii	strictly lanceolate	more or less acutely rounded	28–48	7.0–8.5	10–11	28–30	Levkov et al. 2007
N. weberi	elliptic-lanceolate to broadly lanceolate	obtusely rounded	29–57	7.3–10.3	9–10	ca. 24	Bahls 2012

N. cryptocephala differs from *N. winoniformis* sp. nov. by having lineolae difficult to resolve in LM (40–44/10 μm) and denser striae (14–18/10 μm), no external longitudinal grooves are formed in *N. cryptocephala* and the accessory rib is not unilaterally widened internally (Lange-Bertalot 2001; Jüttner et al. 2020). *N. krammerae* has higher lineolae density (28–31/10 μm) and straight central pores (Lange-Bertalot 2001). *N. densilineolata* possesses non protracted valve ends, no external longitudinal grooves, smaller central area, striae not distinctly convergent at the valve ends, central pores that are bent to the secondary side and denser lineolae (27–30/10 μm) (Lange-Bertalot 2001). *N. wildii* has a smaller central area, coarser striae (11.0–12.5/10 μm), lineolae difficult to resolve in LM, central pores that are bent to the secondary side, and no external longitudinal grooves are formed (Lange-Bertalot 2001). *N. insulsa* has denser striae (14–16/10 μm) and a rather linear-lanceolate valve outline (Metzeltin, Lange-Bertalot 1998). *N. oetzvallensis* has coarser striae (10.5–12.5/10 μm), denser areolae (28–30/10 μm), no external longitudinal grooves and its central pores are broadly expanded and hooked to secondary side (Werum and Lange-Bertalot 2004). *N. winona* differs by gradually attenuated valve ends and proximal raphe ends deflected to the secondary valve side (Bahls 2012). Though *N. praeterita* is quite similar to *N. winoniformis* in LM appearance (Lange-Bertalot 2001), it differs clearly under SEM by the structure of external proximal raphe endings, possessing small projections and larger insertions, and by the absence of distinct external longitudinal grooves.

At the moment *Navicula winoniformis* sp. nov. is abundant and common in rivers and waterbodies of Khánh Hòa Province in Vietnam. Additionally, this species was found in Lâm Đồng Province, neighboring the previous one. We also found this species in a northern province in Vietnam – Lào Cai. Possibly this species is distributed in China too. We found this species only in the River Nam Lik in Laos. We believe that this species is widespread in freshwater systems of Vietnam, Laos and, possibly, China. Additionally, the species was found in Korea as *Navicula gottlandica* Grunow (Lee 2012).

Navicula sparsilineolata sp. nov. described herein, shows some similarity with species such as *Navicula pseudolanceolata* Lange-Bertalot, 1980, *Navicula oppugnata* Hustedt, 1945, *Navicula johncarteri* D.M. Williams, 2001 (syn. *N. concentrica* Carter in Carter & Bailey-Watts, 1981), *Navicula trophicatrix* Lange-Bertalot in Lange-Bertalot & Metzeltin, 1996, *Navicula sancti-naumii* Levkov & Metzeltin, 2007, *Navicula weberi* Bahls, 2012 (Table 3).

Navicula pseudolanceolata differs from *N. sparislineolata* by having denser lineolae (22.0–25.7/10 μm, Lange-Bertalot 2001; Chudaev and Gololobova 2016) and simple drop-like central pores without any projections (Chudaev and Gololobova 2016, pl. 202, fig. 19). *Navicula oppugnata* is characterized by denser lineolae (c. 24/10 μm), and more obtusely rounded valve ends, raphe-sternum appears not elevated externally in light micrographs (Lange-Bertalot 2001) and these features differentiate it from our new species. *Navicula johncarteri* differs from *N. sparislineolata* by possessing wider valves (9–12 μm) with finer lineolae (c. 25/10 μm) and central pores without larger triangular insertions (as *N. concentrica* J. Carter in Carter & Bailey-Watts; see Lange-Bertalot 2001, pl. 72, fig. 6). Though *Navicula trophicatrix* is similar to *N. sparsilineolata*

in external central pores structure, it has less curved terminal fissures (Lange-Bertalot 2001, pl. 66, fig. 1), denser striae (11–13/10 μm) and lineolae (21–24/10 μm) and rather rhombic-lanceolate than lanceolate valve outline (Lange-Bertalot 2001). In *Navicula sancti-naumii* central pores have only small proximal projections and no external longitudinal grooves are formed (Levkov et al. 2007, pl. 47, figs 3–5); areola density in this species is higher (28–30/10 μm), all features that differentiate this species from *N. sparislineolata*. *Navicula weberi* also have denser lineolae (c. 24/10 μm) and striae are distinctly convergent at valve ends (Bahls 2012), two features that distinguish this diatom from *N. sparislineolata*. *Navicula sparsilineolata* sp. nov. was found in two waterfalls in Laos. These waterfalls are 150 km from each other and situated in one Province, namely Champasak. It is possible that this species has more widespread distribution in Southeast Asia.

Description of these two new species shows that the diatom flora of Southeast Asia is a site of biodiversity discovery. The two species described herein are different from some other taxa previously described by us in that they are smaller and lack such prominent morphological features (see Kulikovskiy et al. 2020a,b). *Navicula babeiensis* Chudaev, Glushchenko, Kulikovskiy & Kociolek and *Navicula pseudokuseliana* Chudaev, Glushchenko, Kulikovskiy & Kociolek are smaller and have no special morphological peculiarities like the two species described here (Kulikovskiy et al. 2021). Two previously described species, *Navicula gogorevii* Chudaev, Glushchenko, Kulikovskiy & Kociolek and *Navicula davidovichii* Chudaev, Glushchenko, Kulikovskiy & Kociolek, are characterized by having large valves and large areolae in the striae. *Navicula davidochii* is very interesting and characterized by having a valve shape that is more typical for the genus *Pinnularia* Ehrenberg. Even within the genus *Navicula sensu stricto* we see a diversity of ultrastructural features. And representatives with these different morphologies are present in Southeast Asia. More work is needed to sort out the morphological diversity within *Navicula*, to understand the phylogenetic relationships of these morphological groups, and to establish whether there are biogeographic patterns that correspond to the relationships.

In the book "The diatoms of Southeast Asia" we included widespread taxa from Southeast Asia (Glushchenko et al. 2021). *Navicula* species were detected from three countries including Vietnam, Laos and Cambodia. Widespread taxa are *Navicula escambia* (Patrick) Metzeltin & Lange-Bertalot, 2007, *N. simulata* Manguin 1942, *N. nielsfogedii* Taylor & Cocquyt in Taylor, Cocquyt & Mayama, 2016, *N. heimansioides* Lange-Bertalot, 1993, *N. globuliferiformis* Lange-Bertalot 1993, *N. gondwana* Lange-Bertalot, 1993, *N. tripunctata* (O.F. Müller) Bory, 1822, *N. rostellata* Kützing, 1844, *N. subrhynchocephala* Hustedt, 1935, *N. ingapirca* U. Rumrich & Lange-Bertalot in U. Rumrich, Lange-Bertalot & M. Rumrich, 2000, *N. recens* (Lange-Bertalot) Lange-Bertalot in Krammer & Lange-Bertalot, 1985, *N. radiosa* Kützing, 1844, *N. avenacea* (Rabenhorst) Brébisson ex Grunow in Schneider, 1878, *N. angusta* Grunow, 1860, *N. namibica* Lange-Bertalot & U. Rumrich in Lange-Bertalot, 1993, *N. caterva* Hohn & Hellerman, 1963, *N. vandamii* Schoeman & Archibald, 1987, *N. germainii* Wallace, 1960, *N. amphiceropsis* Lange-

Bertalot & U. Rumrich in U. Rumrich, Lange-Bertalot & M. Rumrich, 2000, *N. quasidisjuncta* Lange-Bertalot & U. Rumrich in U. Rumrich, Lange-Bertalot & M. Rumrich, 2000, *N. electrolytifuga* Lange-Bertalot & U. Rumrich in U. Rumrich, Lange-Bertalot & M. Rumrich, 2000 (Glushchenko et al. 2021).

As evident from the above list of known taxa, Southeast Asia includes many species described from other areas of the Southern Hemisphere, mainly from South America (Rumrich et al. 2000). *N. nielsfogedii* is a widespread taxon on the basis of its morphospecies taxonomy (Chudaev et al. 2020). Possibly, it is cryptic species that can be evident on the basis of molecular investigation in the future. However, if we use molecular methods we can find cryptic speciation. This occurs when some small morphological features (mainly in valve dimensions and shape) are evident in different populations (Chudaev et al. 2020; Glushchenko et al. 2021). Cryptic speciation is a phenomenon that is known in diatoms (Mann 1999); however we do not know precisely how extensively it occurs in different groups of diatoms (Maltsev et al. 2021). Cryptic speciation was shown in the genus *Navicula* by Poulíčková et al. (2010) on the basis investigation of *Navicula cryptocephala*. The same situation can be detected for *N. electrolytifuga* with populations from Laos, Cambodia and Vietnam (see Glushchenko et al. 2021: Pl. 86). Species such as *N. heimansioides*, *N. tripunctata*, *N. rostellata*, *N. subrhynchocephala*, *N. radiosa* are widespread across the Northern and Southern Hemispheres and found in different types of fresh waterbodies (Lange-Bertalot 2001; Kulikovskiy et al. 2016). Molecular investigation of these taxa will be important for the future research of their relationships and use in water quality analysis.

Acknowledgements

The publication is based on research carried out with financial support provided by the Russian Science Foundation (19-14-00320) for M.K., A.G., I.K. (LM, sample preparation for SEM) and by framework of the state assignment (theme No. 121041200194-7) for finishing the manuscript. Samples were collected during expeditions organized and permitted by the Joint Russian-Vietnam Tropical Centre, Ecolan 3.2 theme. SEM studies were carried out at the Shared Research Facility 'Electron microscopy in life sciences' at Moscow State University (Unique Equipment 'Three-dimensional electron microscopy and spectroscopy'). The research for D.Ch. was carried out as part of the Scientific Project of the State Order of the Government of Russian Federation to Lomonosov Moscow State University No. 121032300080-0 (for SEM).

References

Bahls LL (2012) Seven new species in *Navicula* sensu stricto from the Northern Great Plains and Northern Rocky Mountains. Nova Hedwigia 141: 19–38.

Carter JR, Bailey-Watts AE (1981) A taxonomic study of diatoms from standing freshwaters in Shetland. Nova Hedwigia 33(3/4): 513–630.

Chudaev DA, Gololobova MA (2016) Diatoms of Lake Glubokoe (Moscow Region). Moscow, KMK, 447 pp. [in Russian]

Chudaev DA, Glushchenko AM, Blagoveshchenskaya EY, Karthick B, Kulikovskiy MS (2018) Morphology and taxonomy of *Navicula escambia* and *N. simulata* (Bacillariophyceae). Nova Hedwigia. Beiheft 147: 43–57. https://doi.org/10.1127/nova-suppl/2018/006

Chudaev DA, Jüttner I, Glushchenko AM, Kulikovskiy MS, Gurung S, Williams DM (2020) On the geographical distribution of *Navicula nielsfogedii* J.C. Taylor & Cocquyt. Diatom Research 35(2): 185–192. https://doi.org/10.1080/0269249X.2020.1758794

Glushchenko AM, Genkal SI, Kulikovskiy MS (2016) *Aulacoseira konstantinovii* sp. nov. and *Aulacoseira krylovii* sp. nov. – two new centric diatoms from South east Asia. Diatom Research 31(4): 367–378. https://doi.org/10.1080/0269249X.2016.1253617

Glushchenko AM, Kulikovskiy MS, Okhapkin AG, Kociolek JP (2017) *Aneumastus laosica* sp. nov. and *A. genkalii* sp. nov. – two new diatom species from Laos (Indochina) with comments on the biogeography of the genus. Cryptogamie. Algologie 38(3): 183–199. https://doi.org/10.7872/crya/v38.iss3.2017.183

Glushchenko AM, Kulikovskiy MS, Dorofeyuk NI, Kociolek JP (2018) New species and combinations in the genus *Eunotia* Ehrenberg 1837 (Bacillariophyceae: Eunotiaceae) from waterbodies of Southeastern Asia. Nova Hedwigia. Beiheft 147: 69–103. https://doi.org/10.1127/nova-suppl/2018/009

Glushchenko AM, Kuznetsova IV, Kociolek JP, Kulikovskiy MS (2019) *Karthickia verestigmata* gen. et sp. nov. – an interesting diatom with frustular morphology similar to several different cymbelloid diatom genera. Phycologia 58(6): 605–613. https://doi.org/10.1080/00318884.2019.1626605

Glushchenko AM, Kociolek JP, Kuznetsova IV, Kulikovskiy MS (2020) Four new species from the diatom genus *Kobayasiella* Lange-Bertalot from waterbodies of Southeast Asia. Diatom Research 35(4): 363–376. https://doi.org/10.1080/0269249X.2020.1842252

Glushchenko AM, Kuznetsova IV, Kulikovskiy MS (2021) The Diatoms of Southeast Asia. Yaroslavl, Filigran, 320 pp. [in Russian]

Jüttner I, Chudaev DA, Kusber W-H (2020) Re-examination of the type materials of *Navicula exilis* and *Navicula cryptocephala* (Naviculaceae, Bacillariophyceae). Phytotaxa 472(2): 123–134. https://doi.org/10.11646/phytotaxa.472.2.3

Kezlya EM, Glushchenko AM, Kociolek JP, Maltsev YeI, Martynenko NA, Genkal SI, Kulikovskiy MS (2020) *Mayamaea vietnamica* sp. nov.: A new, terrestrial diatom (Bacillariophyceae) species from Vietnam. Algae - Korean Phycological Society 35(4): 325–335. https://doi.org/10.4490/algae.2020.35.11.23

Kociolek JP, Hamsher SE, Kulikovskiy MS, Bramburger A (2017) Are there species flocks in freshwater diatoms? A review of past reports and a look to the future. Hydrobiologia 792(1): 17–35. https://doi.org/10.1007/s10750-016-3075-1

Kociolek JP, Williams DM, Stepanek J, Liu Q, Liu Y, You Q, Karthick B, Kulikovskiy MS (2019) Rampant homoplasy and adaptive radiations in pennate diatoms. Plant Ecology and Evolution 152(2): 131–141. https://doi.org/10.5091/plecevo.2019.1612

Kulikovskiy MS, Lange-Bertalot H, Metzeltin D, Witkowski A (2012) Lake Baikal: Hotspot of endemic diatoms I. Iconographia Diatomologica 23: 7–607.

Kulikovskiy MS, Glushchenko AM, Kociolek JP (2015) The diatom genus *Oricymba* in Vietnam and Laos with description of one new species, and a consideration of its systematic placement. Phytotaxa 227(2): 120–134. https://doi.org/10.11646/phytotaxa.227.2.2

Kulikovskiy MS, Glushchenko AM, Genkal SI, Kuznetsova IV (2016) Identification book of diatoms from Russia. Yaroslavl, Filigran, 804 pp. [in Russian]

Kulikovskiy MS, Glushchenko AM, Kuznetsova IV, Kociolek JP (2018) Description of the new freshwater diatom genus *Okhapkinia* gen. nov. from Laos (Southeast Asia), with notes on family Sellaphoraceae Mereschkowsky 1902. Fottea, Olomouc 18(1): 120–129. https://doi.org/10.5507/fot.2017.021

Kulikovskiy MS, Maltsev YeI, Andreeva SA, Glushchenko AM, Gusev ES, Podunay YuA, Ludwig TV, Tusset E, Kociolek JP (2019) Description of a new diatom genus *Dorofeyukea* gen. nov. with remarks on phylogeny of the family Stauroneidaceae. Journal of Phycology 55(1): 173–185. https://doi.org/10.1111/jpy.12810

Kulikovskiy MS, Chudaev DA, Glushchenko AM, Kuznetsova IV, Kociolek JP (2020a) New diatom species *Navicula davidovichii* from Vietnam (Southeast Asia). Phytotaxa 452(1): 83–91. https://doi.org/10.11646/phytotaxa.452.1.8

Kulikovskiy MS, Chudaev DA, Glushchenko AM, Kuznetsova IV, Krivova ZV, Kociolek JP (2020b) *Navicula gogorevii* – a new, large-celled diatom species from Vietnam (Southeast Asia). Phytotaxa 428(1): 60–66. https://doi.org/10.11646/phytotaxa.428.1.6

Kulikovskiy MS, Chudaev DA, Glushchenko AM, Kuznetsova IV, Frolova LA, Kociolek JP (2021) Two new species of the diatom genus *Navicula* Bory (Bacillariophyceae) from Vietnam (Southeast Asia). Diatom Research 36(1): 63–75. https://doi.org/10.1080/0269249X.2020.1853608

Lange-Bertalot H (2001) *Navicula* sensu stricto. 10 Genera separated from *Navicula* sensu lato. *Frustulia.* Diatoms of Europe 2: 1–526.

Lee JH (2012) Algal flora of Korea. Volume 3, Number 8. Chrysophyta: Bacillariophyceae: Pennales: Raphidineae: Naviculaceae. Freshwater diatoms VI. National Institute of Biological Resources, Incheon, 56 pp.

Levkov Z, Krstic S, Metzeltin D, Nakov T (2007) Diatoms of Lakes Prespa and Ohrid, about 500 taxa from ancient lake system. Iconographia Diatomologica 16: 1–613.

Liu Y, Kociolek JP, Glushchenko AM, Kulikovskiy MS, Fan Y (2018) A new genus of Eunotiales (Bacillariophyta, Bacillariophyceae: Peroniaceae), *Sinoperonia*, from Southeast Asia, exhibiting remarkable phenotypic plasticity, and evidence for another lineage of monorapahid diatoms. Phycologia 57(2): 147–158. https://doi.org/10.2216/17-21.1

Maltsev YeI, Maltseva SY, Kociolek JP, Jahn R, Kulikovskiy MS (2021) Biogeography of the cosmopolitan terrestrial diatom *Hantzschia amphioxys* sensu lato based on molecular and morphological data. Scientific Reports 11(1): e4266. https://doi.org/10.1038/s41598-021-82092-9

Mann DG (1999) The species concept in diatoms. Phycologia 38(6): 437–495. https://doi.org/10.2216/i0031-8884-38-6-437.1

Metzeltin D, Lange-Bertalot H (1998) Tropical diatoms of South America I: About 700 predominantly rarely known or new taxa representative of the neotropical flora. Iconographia Diatomologica 5: 1–695.

Poulíčková A, Veselá J, Neustupa J, Škaloud P (2010) Pseudocryptic diversity versus cosmopolitanism in diatoms: A case study on *Navicula cryptocephala* Kütz. (Bacillariophyceae) and morphologically similar taxa. Protist 161(3): 353–369. https://doi.org/10.1016/j.protis.2009.12.003

Rimet F, Gusev ES, Kahlert M, Kelly M, Kulikovskiy MS, Maltsev YeI, Mann DG, Pfannkuchen M, Trobajo R, Vasselon V, Zimmermann J, Bouchez A (2019) Diat.barcode, an open-access curated barcode library for diatoms. Scientific Reports 9(1): e15116. https://doi.org/10.1038/s41598-019-51500-6

Rumrich U, Lange-Bertalot H, Rumrich M (2000) Diatoms of the Andes. From Venezuela to Patagonia/Tierra del Fuego and two additional contributions. Iconographia Diatomologica 9: 1–673.

Werum M, Lange-Bertalot H (2004) Diatoms in springs, from Central Europe and elsewhere under the influence of hydrogeology and anthropogenic impages. Iconographia Diatomologica 13: 1–417.

Witkowski A, Kulikovskiy MS, Nevrova EL, Lange-Bertalot H, Gogorev RM (2010) The genus *Navicula* in ancient basins. I. Two novelties from the Black Sea. Plant Ecology and Evolution 143(3): 307–317. https://doi.org/10.5091/plecevo.2010.421